



REGULAR ARTICLE

SCREENING OF CRUDE EXTRACT OF FLAVONOIDS OF *MORINGA OLEIFERA* AGAINST BACTERIA AND FUNGAL PATHOGEN

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SUMMARY

Crude extracts were prepared from the flowers and callus of *Moringa oleifera* using ethanol extract and screened for their antimicrobial activity against some bacteria and fungal pathogen by paper disc method. The tested gram positive bacterial strains were *Bacillus subtilis* and *Staphylococcus aureus*, gram negative bacterial strains were *Escherichia coli*, *Klebsiella pneumoniae* and fungal pathogen *Candida albicans*. Although flavonoids are present in all parts of *Moringa oleifera*, maximum amount was observed in flowers. Hence soxhlet extract of 5 grams of powdered flowers and unorganized tissues of *M. oleifera* were screened for their antimicrobial activity. Among the flowers and unorganized tissue tested, the ethanol extract of callus exhibited higher antimicrobial activity when compared to the floral extract.

Key words: Antimicrobial, Antifungal, *Moringa oleifera*, Soxhlet extract, Ethanol extract

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1. Introduction

Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents, with general as well as specific antimicrobial activity (3, 4, 5). There are several reports on the presence of anti-microbial compounds in various plant parts like leaves, bark, fruit, root and flowers (2, 6). A number of plants have been screened for their antimicrobial properties especially due to the presence of phenolic compounds like flavonoids. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. In recent years, secondary plant metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.

India is the largest producer of *Moringa* with an annual production of 1.1 to 1.3 million tones of tender fruits from an area of 380 km². It is a perennial softwood tree with timber. It is also used as a folk medicine in India (1). All parts of the *Moringa* tree are

edible and have long been consumed by humans. *Moringa oleifera* is versatile medicinal plant having variety of medicinal uses. It is recognized for its antispasmodic, anti-inflammatory, diuretic, abortifacient, emmenagogue and ecboic properties and useful in treatment of hysteria, tumors, leucoderma, biliousness etc. (9, 15).

The main objective of this study was to speculate the antimicrobial potential of *Moringa oleifera* and to encourage attempts to commercially produce plant products from plant tissue culture.

2. Materials and Methods

The crude extracts of flavonoids of flowers and callus of *Moringa oleifera* were screened for their antimicrobial activity against some Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* and fungal pathogen *Candida albicans* by paper disc method.

The *Moringa oleifera* flowers were collected from the fields and taken to Department for Botany, Govt. Dungar College, Bikaner for identification and evaluation of antimicrobial activity.

Unorganized cultures with profuse callusing were established using seeds as explants, on Murashige and Skoogs medium supplemented with 1.5mg/L BAP + 1.5mg/L 2,4-D. These cultures were maintained for a period of six months by frequent

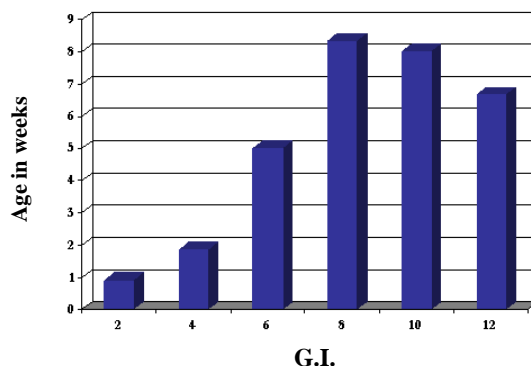
subculturing at interval of 6 to 8 weeks at 26 + 1°C, 55% relative humidity and diffused light conditions (3000 lux). The growth indices (GI) were calculated at different time intervals of 2,4,6,8 and 10 weeks (Table and plate no.1) using the formula given below. Cultures at the maximum growth indices were harvested, dried and analyzed for antimicrobial activity.

$$GI = \frac{\text{Final fresh weight of tissue} - \text{Initial fresh weight of tissue}}{\text{Initial fresh weight of tissue}}$$

Table no. – 1: Growth indices of static cultures of *M. oleifera* at different age intervals

Age of Tissue	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks
Growth index of unorganized tissue	0.92±0.06	1.86±0.08	5.01±0.13	8.34±0.35	8.02±0.27	6.68±0.18

Plate no. – 1: Growth indices of static cultures of *M. oleifera* at different age intervals



Extraction of the sample

Five grams of powdered flowers and unorganized tissues (harvested at maximum growth age of eight weeks) of *M. oleifera* were separately Soxhlet extracted by method of Subramanian and Nagrajan (1969), in 80% ethanol (100 ml/g.d.w.) on a water bath for 24 hrs to extract flavonoids in them. This crude extract dried and dissolved in minimal amount of distilled water was used as test samples.

Disc preparation and antimicrobial sensitivity test

The bacterial cultures were maintained on nutrient broth (10% peptone, 0.5% Lab-Lemco and 0.5% NaCl, pH adjusted at 7.5) where as cultures of *Candida albicans* were maintained on Sabouraud Liquid Medium

(1% peptone, 4% glucose and adjusted to pH 5.8). These microorganisms were allowed to grow at a temperature of 35-37°C. The inoculum used for screening studies was prepared by adjusting the concentration of microorganisms in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm. 40% transmittance was used in case of bacteria while 65% transmittance in case of *Candida albicans*. Pairs of petriplates were washed, rinsed with sterile distilled water, dried, wrapped in tin foil and kept in oven at 100°C for 18 hours to sterilize. For testing antimicrobial activity against bacteria, 10ml of growth medium and 4 ml of inoculum whereas for *Candida albicans*, 10 ml of growth medium and 6.5 ml of inoculum were mixed and poured in separate sterilized pairs of petriplates. Each mixture was

thoroughly shaken to ensure uniform distribution of inoculum. Experiment was carried out in five replicates. Paper discs measuring 6 mm diameter, which absorb about 0.1 ml of the solution were employed for test in test samples. All the test petriplates were kept at 5°C for 40-50 minutes so as to allow the diffusion of the substances and then incubated at 35-37°C for 18 hours. The inhibition zones formed by the crude extract

of flavonoids (test samples) were measured including diameter of paper disc (6 mm).

3. Results

The antimicrobial activity of *M. oleifera* floral and callus extract was assayed by paper disc method against some bacteria and fungal pathogen. Table and Plate 2 summarize the microbial growth inhibition of ethanol extract of the screened plant part (flowers) and unorganized callus.

Table no. -2: Antimicrobial activity of crude extract of flavonoids of *Moringa oleifera*

CRUDE EXTRACT	GRAM+VE BACTERIA		GRAM -VE BACTERIA		FUNGAL PATHOGEN
	BS	SA	EC	KP	CA
Flowers	10.5	9.0	8.6	8.0	6.5
Callus	10.8	9.3	8.8	8.5	6.7

Values represent diameter of zone of inhibition in mm including diameter of paper disc (6 mm). Experiment was repeated five times. The values represent the average diameter.

SA = *Staphylococcus aureus*, EC = *Escherichia coli*, BS = *Bacillus subtilis*, KP = *Klebsiella pneumoniae*, CA = *Candida albicans*.

Plate 2: Antimicrobial activity of crude extract of flavonoids of *Moringa oleifera*

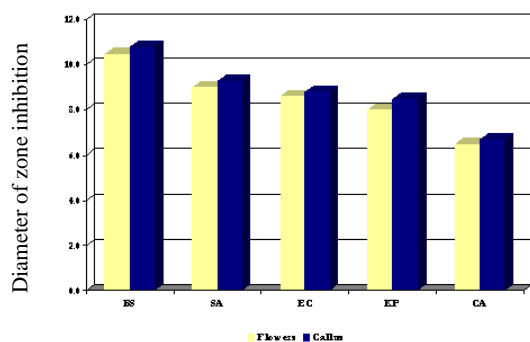


Fig. 1: Antimicrobial screening of crude extract of flavonoids against gram +ve bacteria

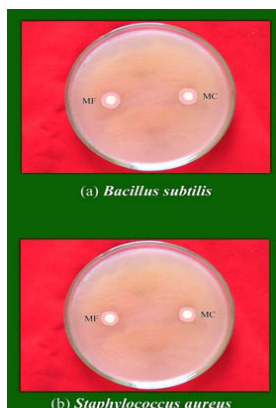


Fig. 2: Antimicrobial screening of crude extract of flavonoids against gram -ve bacteria

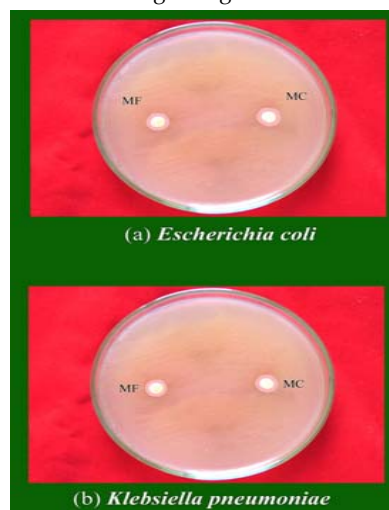
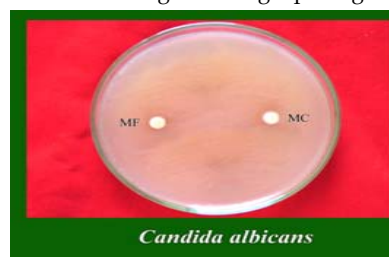


Fig. 3: Antimicrobial screening of crude extract of flavonoids against fungal pathogen



The *M. oleifera* flowers showed maximum antimicrobial response against Gram-positive bacterium *Bacillus subtilis* (diameter of zone of inhibition 10.5 mm). Unorganized tissue had more amount of flavonoids than maximum reported in flowers, hence the zone of inhibition formed against *Bacillus subtilis* was 10.8mm. Zone of inhibition formed against Gram +ive bacterium *Staphylococcus aureus*, floral extract and unorganized tissue extract was 9.0 mm and 9.3 mm respectively. It showed that the extract of *M. oleifera* has little less activity against *Staphylococcus aureus*. Antimicrobial activity of floral and callus extract calculated against Gram -ve bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) was 8.6 mm and 8.8 mm (against *Escherichia coli*) while 8.0 mm and 8.5 mm (against *Klebsiella pneumoniae*) respectively. Minimum activity of *Moringa* flavonoids was reported against fungal pathogen *Candida albicans* 6.5 mm with floral extract and 6.7 mm with callus extract).

4. Discussion

In the present scenario of emergence of multidrug resistance to human pathogenic infections, it has become very necessary to search for new antimicrobial substances from other sources such as plants (7, 8, 10, 16). It will lead to the development of a phytomedicine to act against microbes (11, 12, 13, 14). Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. *M. oleifera* is highly valued plant, with impressive range of medicinal uses and high nutritional value. A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses is developing to support at least some of the claims *Moringa* preparations known to have antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities as cited in the scientific literature. Further purification of compounds can be done and the compounds may be subjected for animal studies.

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